Salivary gland involvement in rheumatoid arthritis and its relationship to induced oxidative stress

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\textbf{Objectives.} The objective of the present study was to analyse salivary gland and free radical involvement in rheumatoid arthritis (RA).

\textbf{Methods.} Thirty-four consenting RA patients (rheumatoid factor-positive) and 18 healthy controls, matched in age and gender, participated in the study. Plasma and saliva samples were harvested and subjected to compositional analysis and various free radical-related tests.

\textbf{Results.} The mean salivary flow rate was lower in the RA patients than in the control group, whereas all plasma and salivary antioxidants were increased. Mean values of plasma malondialdehyde and ceruloplasmin were higher in the RA patients.

\textbf{Conclusions.} The effects of RA on salivary gland flow rates and antioxidant compositional parameters may be of great importance for the further elucidation of the role of free radicals in RA pathogenesis and for its general diagnosis and evaluation. The demonstrated correlation between the altered salivary parameters and the severity of the disease may indicate that evaluation of the salivary status of RA patients is warranted.

\textbf{KEY WORDS:} Saliva, Antioxidants, Rheumatoid arthritis, Total antioxidant status, Peroxidase, Uric acid, Malondialdehyde, Ceruloplasmin, Creatinine.

Rheumatoid arthritis (RA) is a heterogeneous disease with a spectrum of clinical severity ranging from mild arthritis to a crippling joint disorder with internal organ involvement. The pathogenesis of RA is characterized by prolonged, chronic inflammation of the synovial membrane accompanied by morphological alterations and the recruitment of mononuclear and polymorphonuclear cells into the synovial fluid\textsuperscript{1, 2}. However, in recent years it has become clear that, besides the immunological reaction, there is another biological process, based on the injurious activity of free radicals\textsuperscript{3, 4}, playing a major role in the pathogenesis. For example, it was demonstrated that the level of free radical-induced damage to proteins in the synovial fluid was twice as high in RA patients\textsuperscript{2, 5}. Furthermore, an increase in the \textit{in vivo} generation of oxidants and lipid peroxidation products was demonstrated in the plasma of RA patients, which correlated with the antioxidant levels\textsuperscript{6}. Thus, the levels of plasma malondialdehyde (MDA), an index of lipid peroxidation, and of the antioxidants ceruloplasmin (CP) and erythrocyte glutathione were found to be significantly higher in RA patients than in healthy controls\textsuperscript{7, 8}. Ceruloplasmin is considered the principle plasma and synovial antioxidant in RA, being responsible for up to 70\% of the protective capacity against superoxide free radicals\textsuperscript{9}, which have been shown to be directly related to...
the pathogenesis of the inflamed joint in RA and the related increases in lipid peroxidation, ascorbate depletion and hyaluronate degradation [10]. Moreover, it was also found that individuals with innately low levels of protecting antioxidants in their plasma, such as vitamins A and E, β-carotene and selenium, are also at greater risk of developing RA [11, 12]. The two most often suggested mechanisms for the increased incidence and activity of free radicals in RA joints are: (i) the production of various free radicals, such as superoxide, hydroxyl and hypochlorus by the invading phagocytes [13, 14]; and (ii) an increase in the intra-articular pressure above the synovial capillary perfusion pressure, causing intra-articular hypoxia. On cessation of exercise of the RA-inflamed joint, an injurious reperfusion mechanism occurs, resulting in oxidative damage to lipids and immunoglobulin (Ig) within the joint [15].

Interestingly, although salivary gland involvement in RA has been known for a long time, it did not draw much attention, either from clinicians or researchers. In 1978, Sullivan et al. [16] reported that 58 of 100 unselected RA out-patients had reduced lachrymal and/or salivary secretion. Morphological studies revealed that the minor salivary glands of RA patients were highly infiltrated with lymphocytes, B cells predominating over T cells and with a higher ratio of helper T cells to suppressor T cells, and that the alterations also included fibrosis, acinar atrophy and lymphoplasma cell sialadenitis [17, 18]. Moreover, Grevers et al. [18] reported that 20% of the RA patients in their studies also suffered from secondary Sjögren’s syndrome, while immunoserological analysis of these patients demonstrated abnormal titres of circulating immune complexes of 71% of patients, the presence of SSA and SSB antibodies in 32%, and the presence of antinuclear antibodies in 27%. Interestingly, in a similar study it was reported that anti-salivary duct antibodies were demonstrated in the serum of RA patients with or without accompanying secondary Sjögren’s syndrome but not in patients with primary Sjögren’s syndrome [19].

In spite of the relatively large amount of accumulated evidence that the salivary glands are profoundly affected in RA, there is no available study analysing the antioxidant profile of the secreted saliva of these patients. This constitutes a major omission, as such an analysis may contribute in three ways.

First, it is well established that, to a large extent, saliva composition represents plasma composition, and accordingly its analysis is warranted. Such an analysis is easy, non-invasive, cheap and patient-friendly, and thus it may replace plasma analysis of antioxidants, which in turn may be of importance in monitoring the severity of the condition and/or the success of its treatment.

Secondly, salivary antioxidants play an important physiological role in the oral protective system and may also be important in the gastrointestinal tract after the saliva has been swallowed (as described for salivary epidermal growth factor (EGF), for example). Accordingly, if an altered salivary antioxidant profile in RA patients accompanies reduction in saliva output there may be further injurious effects.

Thirdly, the destructive effect on the salivary glands demonstrated in RA patients may be rendered by a free radicals-related process, and thus such an analysis may contribute to the better understanding of the mechanism.

Materials and methods

Experimental design

Thirty-four consenting rheumatoid factor-positive and otherwise healthy RA patients and 18 healthy controls, matching the patients in age and gender, participated in the study. The severity of RA was assessed with the well-established Health Assessment Questionnaire (HAQ) grading criteria. On the day HAQ grading was determined for each patient, plasma and whole saliva samples were also harvested simultaneously for further analysis [20–22]. Informed patient consent and ethical approval was obtained for the study.

Plasma analysis of ceruloplasmin, malondialdehyde, uric acid and creatinine

Plasma was centrifuged (3000 r.p.m.) immediately after collection, and the supernatant was analysed during the next 12 h. Ravin’s method was used to determine the CP concentration [23]. The method of Slater and Sawyer [24] was used to measure production of malondialdehyde (MDA). Briefly, thiobarbituric acid was used during the reaction to assess the level of induced lipid peroxidation, as there is a direct correlation between the two. Creatinine and uric acid (UA) were determined by immunoturbidimetry with a Hitachi 911 automatic analyser (Hitachi, Tokyo, Japan) using reagents purchased from Roche Diagnostics (Mannheim, Germany).

Sialometry

Unstimulated (resting) whole saliva specimens were obtained in the morning and no oral stimulus was permitted for 90 min prior to collection. The saliva was collected for 10 min, as described previously [25–27]. Collection was onto ice, and the salivary specimens were then frozen at -20°C for analysis. The salivary gland flow rate was expressed as the volume of saliva (in ml) secreted per minute.

Sialochemistry

The sialochemical analysis included peroxidase activity, total antioxidant status (TAS) and UA concentration. All variables were expressed both as values normalized to volume (concentrations) and as total values (secreted into the oral cavity per minute).

The cut-off value of minimum flow rate used for dividing the observations into two subgroups was 0.15 ml/min, which is considered in the literature as the minimum value for healthy individuals [27].

Salivary peroxidase activity

Salivary peroxidase activity was measured according to thionitrobenzoic acid (NBS) assay, as described previously [25]. Briefly, the calorimetric change induced by the reaction between the enzyme and the substrate (dithio-bis-2-nitrobenzoic acid) in the presence of mercaptoethanol was read at a wavelength of 412 nm for 20 s.
Salivary total antioxidant status

TAS was assessed as described previously [27]. Briefly, this assay is based on a commercial kit supplied by Randox (San Francisco, CA, USA) in which metmyoglobin, in the presence of iron, is turned into ferrymoglobin. Incubation of the latter with the Randox reagent ABTS results in the formation of a radical coloured blue-green, which can be detected at 600 nm.

Salivary UA concentration

The concentration of UA was measured with a kit supplied by Sentinel (Milan, Italy) as described previously [26]. In this assay, UA is transformed by uricase into allantoin and hydrogen peroxide, which, under the catalytic influence of peroxidase, oxidizes the chromogen (4-aminophenazone/N-ethyl-methylanilin propan-sulphonate sodic) to form a red compound, the intensity of colour of which is proportional to the amount of UA present in the sample and is read at a wavelength of 546 nm.

Statistical analysis

Results for statistical evaluation were taken from healthy controls and RA patients. The evaluation was also performed after the patients had been divided into subgroups according to the severity of their disease (using HAQ criteria; three subgroups) and according to the severity of salivary involvement (using flow rate values; two subgroups). The plasma (MDA, CP, UA, creatinine) and salivary (flow rate, peroxidase, TAS, UA) variables of the patients were obtained; means and standard errors were computed. Correlations and significances of the plasma variables were determined by Pearson correlation analysis. Differences in mean values of plasma and saliva variables between healthy controls and RA patients were analysed with the two-sample t-test. The sensitivities and specificities of the saliva variables in the healthy control group and the RA patients were computed.

Results

Patient characteristics

The mean age of the controls ± standard errors (n=18; 4 males, 14 females) was 46.6 ± 4.6 yr and that of the RA group was 51.0 ± 2.0 (n=34; 8 males, 26 females) (Table 1). The mean duration of disease since primary diagnosis was 6.28 ± 1.16 yr for these patients. The patients were divided into three subgroups according to well-established HAQ criteria: mildly affected (n=10), moderately affected (n=17) and severely affected (n=7). To delineate the specific effect of RA on salivary gland function, as expressed by flow rate reduction, the patients were divided into two subgroups according to the severity of the reduction in flow rate: mildly affected (n=22) and severely affected (n=12). As mentioned previously, the cut-off value of minimum flow rate used to create the two subgroups was 0.15 ml/min, which was also the minimum flow rate value found in the healthy controls in the current study. The mean HAQ values in these subgroups with mild and severe affection of salivary flow rate were 1.25 ± 0.17 and 1.87 ± 0.21 respectively, and their durations of disease were 5.42 ± 1.11 and 7.85 ± 2.58 yr respectively (Table 2, Fig. 1). A linear correlation was demonstrated between HAQ value and disease duration (r = 0.50; P = 0.006) (Fig. 2).

Plasma variables (ceruloplasmin, UA, MDA, creatinine)

The MDA and CP concentrations in healthy controls were 7.36 ± 0.44 nm/ml and 23.74 ± 1.78 mg/dl respectively. In the RA patients, the mean values of MDA and CP were significantly higher, by 33% (P = 0.0012) and 35% (P = 0.0001) respectively (Table 3). A linear correlation was found between the MDA and CP values (r = 0.7; P = 0.0001) (Fig. 3). The UA and creatinine concentrations in the patients’ plasma were 4.73 ± 0.21

Table 1. Age and gender distributions of healthy controls and RA patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
<th>Age (yr; mean ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>18</td>
<td>14</td>
<td>4</td>
<td>36.6 ± 4.6</td>
</tr>
<tr>
<td>RA (total)</td>
<td>34</td>
<td>8</td>
<td>26</td>
<td>51.0 ± 2.0</td>
</tr>
<tr>
<td>RA (0 &lt; HAQ &lt; 1)</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>49.9 ± 3.0</td>
</tr>
<tr>
<td>RA (1 &lt; HAQ &lt; 2)</td>
<td>17</td>
<td>4</td>
<td>13</td>
<td>50.0 ± 3.1</td>
</tr>
<tr>
<td>RA (2 &lt; HAQ &lt; 3)</td>
<td>7</td>
<td>1</td>
<td>6</td>
<td>59.0 ± 3.9</td>
</tr>
<tr>
<td>RA with mild salivary involvement</td>
<td>22</td>
<td>4</td>
<td>18</td>
<td>51.2 ± 2.7</td>
</tr>
<tr>
<td>RA with severe salivary involvement</td>
<td>12</td>
<td>4</td>
<td>8</td>
<td>52.7 ± 2.7</td>
</tr>
</tbody>
</table>

Table 2. Salivary flow rates and RA activity (HAQ criteria) in healthy controls and in patients with mild vs severe salivary involvement

<table>
<thead>
<tr>
<th>Flow rate (ml/min)</th>
<th>Healthy</th>
<th>RA, mild</th>
<th>RA, severe</th>
<th>Healthy/RA, mild</th>
<th>Healthy/RA, severe</th>
<th>RA, mild/severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.48</td>
<td>0.35</td>
<td>0.10</td>
<td>P = 0.013</td>
<td>Sig</td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>SE</td>
<td>0.05</td>
<td>0.03</td>
<td>0.01</td>
<td>Sig</td>
<td></td>
<td>P = 0.0001</td>
</tr>
<tr>
<td>HAQ</td>
<td>–</td>
<td>1.25</td>
<td>1.87</td>
<td>–</td>
<td>–</td>
<td>P = 0.015</td>
</tr>
<tr>
<td>Mean</td>
<td>–</td>
<td>0.17</td>
<td>0.21</td>
<td>–</td>
<td>–</td>
<td>Sig</td>
</tr>
<tr>
<td>Disease duration (yr)</td>
<td>Mean</td>
<td>5.42</td>
<td>7.85</td>
<td>–</td>
<td>–</td>
<td>P = 0.016</td>
</tr>
<tr>
<td>SE</td>
<td>1.11</td>
<td>2.58</td>
<td></td>
<td>–</td>
<td>–</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Sig, significant; n.s., not significant.
mg/dl ($P = 0.02$ vs controls) and $0.85 \pm 0.03$ mg/dl (not significantly different from controls) respectively (Table 3). A significant linear correlation was demonstrated between UA and creatinine concentrations in the plasma ($r = 0.75$, $P = 0.0001$) (Fig. 4).

**Sialometry**

The mean salivary flow rate of the healthy controls was $0.48 \pm 0.05$ ml/min, and was 46% lower ($P = 0.0001$) in the RA patients (Table 3). There were 22 RA patients (64.7%) with a mild reduction (> 0.15 ml/min) and 12 RA patients (35.3%) with a severe reduction in salivary flow rate (< 0.15 ml/min). The mean flow rates of these two subgroups were $0.35 \pm 0.03$ and $0.10 \pm 0.01$ ml/min respectively (Table 2).

**Sialochemistry**

**Salivary peroxidase activity.** The mean peroxidase activity, normalized to volume, of healthy controls was $480 \pm 132$ U/100 µl; it was 78% higher ($P = 0.02$) in the RA patients (Table 3). Peroxidase activities in the RA subgroups with mildly and severely reduced salivary flow rate were not significantly different from one another but were 70% higher ($P = 0.04$) and 92% higher ($P = 0.032$) respectively than in healthy controls (Table 4, Fig. 5A). The mean total peroxidase activity value of healthy controls was $1.7 \times 10^2 \pm 3.0 \times 10^1$ U/min. The total peroxidase activity values of the RA subgroups with mildly and severely reduced salivary flow rate were significantly different from one another and were 65% higher ($P = 0.036$) and 35% lower ($P = 0.05$) respectively than in the healthy controls (Table 4, Fig. 6A).

**Salivary TAS.** The mean TAS value, normalized to volume, of healthy controls was $1.10 \pm 0.20$ mM/l, and it was 97% higher ($P = 0.003$) in the RA patients (Table 3). The TAS values of the RA subgroups with mildly and severely reduced salivary flow rate were not significantly different from one another but were 81%
higher \( (P = 0.008) \) and 136\% higher \( (P = 0.003) \), respectively than in healthy controls (Table 4, Fig. 5B), a pattern similar to that demonstrated for peroxidase activity. A pattern similar to that found for total peroxidase activity was demonstrated for the total TAS value. Thus, the mean total TAS value of

Table 4. Salivary antioxidants in healthy controls vs RA patients with mild and severe salivary involvement

<table>
<thead>
<tr>
<th></th>
<th>Healthy ((n = 18))</th>
<th>RA, mild ((n = 22))</th>
<th>RA, severe ((n = 12))</th>
<th>Significance of differences between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase (U/100 (\mu)l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>480</td>
<td>816</td>
<td>920</td>
<td>( P = 0.04 ) ( P = 0.032 ) ( P = 0.32 )</td>
</tr>
<tr>
<td>SE</td>
<td>132</td>
<td>130</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>Peroxidase (total) (U/100 ml) ( \times ) (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>170</td>
<td>282</td>
<td>110</td>
<td>( P = 0.036 ) ( P = 0.05 ) ( P = 0.001 )</td>
</tr>
<tr>
<td>SE</td>
<td>30</td>
<td>48</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>TAS (mM/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.10</td>
<td>2.00</td>
<td>2.60</td>
<td>( P = 0.008 ) ( P = 0.003 ) ( P = 0.16 )</td>
</tr>
<tr>
<td>SE</td>
<td>0.20</td>
<td>0.28</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>TAS (total) (mM/l) ( \times ) (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.45</td>
<td>0.72</td>
<td>0.32</td>
<td>( P = 0.03 ) ( P = 0.15 ) ( P = 0.037 )</td>
</tr>
<tr>
<td>SE</td>
<td>0.07</td>
<td>0.12</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.34</td>
<td>3.37</td>
<td>6.10</td>
<td>( P = 0.48 ) ( P = 0.008 ) ( P = 0.008 )</td>
</tr>
<tr>
<td>SE</td>
<td>0.46</td>
<td>0.47</td>
<td>1.20</td>
<td></td>
</tr>
<tr>
<td>UA (total) (mg/dl) ( \times ) (ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.50</td>
<td>1.18</td>
<td>0.70</td>
<td>( P = 0.14 ) ( P = 0.016 ) ( P = 0.075 )</td>
</tr>
<tr>
<td>SE</td>
<td>0.20</td>
<td>0.20</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

n.s., not significant.
healthy controls was $0.45 \times 10^{-3} \pm 0.07 \times 10^{-3}$ mm/min. The total TAS values of the RA subgroups with mildly and severely reduced salivary flow rate were significantly different from one another and were 60% higher ($P = 0.03$) and 29% lower (not significant) respectively than in the healthy controls (Table 4, Fig. 6B).

**Salivary UA concentration.** The mean UA concentration, normalized to volume, of healthy controls was $3.34 \pm 0.46$ mg/dl and was 23% higher (not significant) in the RA patients (Table 3). Interestingly, a linear correlation was demonstrated between the UA concentrations in plasma and saliva ($r = 0.60$, $P = 0.005$) (Fig. 7). The UA concentrations of the RA subgroups with mildly and severely reduced salivary flow rate were significantly different from one another ($P = 0.008$); compared with values for healthy controls, these values were similar ($3.37$ mg/dl, not significant) and 80% lower ($P = 0.008$) respectively (Table 4, Fig. 6C). The mean total UA concentration in healthy controls was $1.5 \times 10^{-2} \pm 0.2 \times 10^{-2}$ (mg/min) (Table 2). The UA concentrations of the RA subgroups with mildly and severely reduced salivary flow rate were significantly different from one another and were similar to ($1.18 \times 10^{-2}$ mg/min, not significant) and 54% lower ($P = 0.016$) respectively than values in healthy controls (Table 4, Fig. 6C).

The two most important findings of the present study relate to the effect of RA on the salivary glands on one hand and to the role of oxidative stress in the clinical manifestation of the disease on the other hand. The demonstrated parallel, significant increase in both the plasma antioxidants CP (by 35%) and UA (by 27%) and the lipid peroxidation product MDA (by 33%) in the RA patients is not surprising in the light of the well-established role that free radicals play in the pathogenesis of the disease, and is in accord with previous reports [4, 5, 20]. Accordingly, the linear correlation between the plasma concentrations of CP and MDA in the RA patients demonstrated in the
present study is explainable, and so is the demonstrated linear correlation between the plasma concentrations of UA and creatinine. However, the increase in the antioxidant capacity produced by CP seems unable to cope with the RA-induced oxidative stress, and thus the induced lipid peroxidation is not fully prevented (as indicated by the increase in MDA values).

The significant reduction in salivary gland function (as measured by flow rate) in the RA patients (by 46%, $P=0.0001$) is also not surprising. This result points to the salivary—glands as major target organs of RA. This notion gains further credence by the demonstrated significant correlations between the severity of the RA (according to HAQ criteria) and (i) the reduction in salivary flow rate and (ii) the duration of disease.

The demonstration of RA-induced alterations in the salivary antioxidant system is novel and its related clinical significance seems to be of paramount importance. All plasma and salivary antioxidants increased in RA patients. The mean values, normalized to salivary volume, of peroxidase activity and TAS and UA concentrations were increased by 78, 97 and 23% respectively in these patients. Moreover, in the subgroup of patients ($n=12$) in whom the salivary flow rate was reduced most severely (to $<0.15$ ml/min), these values were increased, by 91, 136 and 82% respectively. This overall increase in salivary antioxidants in RA patients may result from the general increase in plasma antioxidants, previously demonstrated, because to a large extent plasma composition is reflected in saliva composition [27, 28]. However, it may also reflect a similar specific response of the salivary glands to the RA, i.e. up-regulation of the production of specifically salivary antioxidants. It seems that both mechanisms act in concert in RA pathogenesis, in the light of the fact that peroxidase, the most important salivary antioxidant enzyme, is known to be produced specifically in the parotid gland while UA, the most important salivary antioxidant molecule, is plasma-borne [25, 26]. Thus, the high linear correlation between the saliva and plasma concentrations of UA demonstrated here is no surprise. TAS is the sum of all the various salivary antioxidants found in the saliva, regardless of their origin. The fact that the increase in peroxidase was much higher than the increase in UA may suggest that the active role played by the salivary glands themselves in fighting oxidative stress-related pathology in the oral cavity of the RA patients is of major importance.

However, another explanation for the difference in the levels of increased salivary UA vs peroxidase may be based on the specific injurious effect of RA on the salivary glands. Subdividing the RA patients according to the severity of their salivary flow rate reduction revealed that, while peroxidase and TAS were increased in both subgroups in a similar manner (being approximately doubled), the UA concentration was profoundly increased only in the severely affected RA patients (by 82%, $P=0.008$) and not in the mildly affected RA subgroup. This result may have a very interesting mechanistic significance, as it may be that the pathway of UA transduction from the plasma via the parotid parenchymal cells (through some specific channel?) is less sensitive to the injury inflicted by RA, while the acinar cellular machinery responsible for producing and secreting peroxidase (and other salivary enzymes?) is more vulnerable. Accordingly, RA-induced effects on peroxidase and TAS are already observed in mildly affected patients whereas the effect on UA is observed only in severely affected patients.

From the clinical standpoint, it is the total rather than the volume-normalized values of salivary antioxidants which are important, as they reflect the total antioxidant capacity secreted into the oral cavity. In order to assess these values, one must consider the reduction in volume of the secreted saliva, not only its antioxidant concentrations. It was found that all three salivary antioxidant parameters analysed presented a pattern of response similar to that of the severity of the RA. The total peroxidase activity, TAS values and UA concentrations were significantly increased in the mildly affected subgroup (by 35–51%) and significantly reduced in the severely affected subgroup (by 45–80%).

Obviously, as explained previously, this reflects the sum of the RA-induced effects as elicited in the oral cavity. Thus, the increase in salivary antioxidants dominates the outcome in mildly affected patients while the flow rate reduction dominates in severely affected patients. This finding may be of paramount importance in the light of the presumably major importance of the salivary antioxidant capacity in fighting various pathologies in the oral cavity (and probably, after the saliva is swallowed, also in the gastrointestinal tract) [26, 27]. For example, the effect of cigarette smoke on salivary peroxidase was only recently found to be devastating (70–80% reduction in enzyme activity), which is in good accord with the increase in the incidence of oral diseases, such as gingivitis and squamous cell carcinoma in cigarette smokers [25, 29]. These results suggest that RA patients in whom there is both a severe reduction in the salivary flow rate in general and in the total amount of salivary antioxidants secreted into the oral cavity in particular are significantly less well protected. The mutual increase in salivary antioxidant parameters (when normalized to volume) in the mildly affected RA subgroup indicates that in these patients the salivary glands are coping with the injurious effects of the disease. However, when the insult to the salivary glands induced by RA becomes too severe, they can no longer cope.

From the clinical standpoint, it seems reasonable to conclude that severely affected RA patients should be treated both with antioxidants and with saliva substituents and that extra care should be given to the oral cavity. Finally, we think that the profound effects of RA on salivary gland flow rates and antioxidant parameters that we have demonstrated may also be of great importance in the general diagnosis and evaluation of the disease. In this respect it is important to note that the sensitivity and
specification values of all three salivary antioxidants evaluated (peroxidase, UA and TAS) were in the range of 60–85%. The demonstrated correlation between altered salivary parameters and the severity of the disease may indicate that evaluation of the salivary status of RA patients as part of the assessment of their disease activity and severity is warranted.

Acknowledgement
The authors thank Mrs S. Gan for her assistance in the statistical analysis of the results.

Conflict of interest
The authors declare that they have no conflicts of interest.

References